

REMARKS

Incorrect Attorney of Record, Mailing Address, and Docket Number

The first page of Paper No. 10 contains the name and address of an attorney no longer of record in this application and the former attorney's docket number. As the Assignee of this application requested in the Revocation of Original Power of Attorney and Grant of New Power of Attorney filed on December 5, 1995, Applicant again respectfully requests that the PTO update its records so that all future communications regarding this application are mailed to:

Kenneth J. Meyers, Esq.
Finnegan, Henderson, Farabow,
Garrett & Dunner, L.L.P.
1300 I Street N.W.
Washington, D.C. 20001-3315,

Applicant also requests that the PTO use the proper attorney docket number (04270.0015) for this application in all future communications.

Claim Amendments

Reconsideration of this application is respectfully requested.

Applicant has canceled all the pending claims (claims 37-71) and has added new claims 72-101 to further clarify the claimed invention. Upon entry of this amendment, claims 72-101 will be pending in this application. The specification and originally filed claims support new claims 72-101. For example, the specification supports new independent claim 72 as follows:

Claim

72. A method of transferring a gene into an animal, comprising:

Applicant's Disclosure

This invention relates to a technique for altering the level of gene expression which involves the introduction of a genetically engineered cell into a recipient individual. (Page 1, lines 3-6).

In a somatic cell gene delivery system, cells from the patient are removed, cultured in vitro, transfected, and reimplanted. Modifications of the basic scheme include . . . the transfection protocol, and the site of reimplantation.

Several techniques have thus far been developed which offer promise as means for delivering DNA into an individual. (Page 3, lines 13-22).

In the past few years, it has become apparent that the implementation of retroviral based gene delivery systems in humans will face major obstacles, primarily related to properties of retroviruses themselves (Robertson, M., Nature 320:213-214 (1986), Marx, J.L., Science 232:824-825 (1986)). (Page 4, lines 16-21).

Since the infected cells are not characterized before reintroduction, the possibility of a deleterious integration [sic integration] event cannot be eliminated. (Id. at lines 29-31).

An alternative approach for gene therapy involves introducing DNA into a cell by chemical, as opposed to viral, techniques. In this approach, DNA is introduced into a recipient cell by calcium phosphate-mediated transfection. In general, the recipient cells are first removed from an individual

LAW OFFICES

FINNEGAN, HENDERSON,
FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000

and incubated in the presence of a DNA solution containing the gene whose introduction is desired. After the gene has been introduced into the cell, the cell is returned to the individual. (Page 5, lines 8-17).

Figure 1 shows a diagrammatic representation of one embodiment of transkaryotic implantation. Cultured cells are co-transfected with the gene of therapeutic interest and a gene encoding a selectable marker.

* * *

A clonal line possessing the desired expression properties is then introduced into one of a variety of anatomical locations in the host animal, which is itself characterized with respect to expression of the gene of interest. (Page 12, line 22, through page 13, line 65).

Co-Transfect with Therapeutic Gene of Interest . . . Introduce Cells Into Desired Anatomical Location. (Figure 1).

This alteration involves the introduction of a transfected cell which carries either a desired gene sequence or an effector gene sequence into the recipient subject. (Page 15, lines 10-13).

Gene expression may be increased by providing to the subject recipient a transfected cell which contains a gene substantially identical or equivalent to that gene of the recipient subject whose amplified expression is desired. (Id., lines 24-28).

It is possible to increase gene expression in accordance with the

present invention by providing to the recipient subject a transfected cell which contains an "effector gene sequence" (Page 22, lines 9-14).

The transfected cells of the present invention may be introduced into a recipient subject, (Page 37, lines 3-19).

The present invention may be practiced with a transkaryotic cell . . . cultured in vitro and reintroduced into the recipient subject. (Page 37, lines 20-25).

The "subject recipient" with which the present invention may be employed include [sic, includes] animals, as well as humans (Page 15, lines 18-19).

(a) transfecting somatic cells *in vitro* with a DNA sequence,

Somatic cell gene therapy, however, does seem to be a reasonable approach to the treatment and cure of certain disorders in human beings. (Page 3, lines 11-13).

Cultured cells are co-transfected with a gene of therapeutic interest and a gene encoding a selectable marker. Stably transfected cells (Page 12, lines 23-30).

A transkaryotic cell is produced through the *in vitro* introduction of an additional genetic sequence into a cell (Fig. 1). (Page 33, lines 11-13).

Co-Transfect with Therapeutic Gene of Interest (Figure 1).

After transfection, it is preferable to allow the transfected cells to proliferate (Page 36, lines 26-29).

and without a viral vector, wherein the DNA sequence comprises no DNA of retroviral origin,

The present invention may be practiced with a transkaryotic cell derived from a wide number of diverse tissues, as long as the cell is (1) capable of receiving and expressing the introduced gene sequences, and (2) being cultured *in vitro* and reintroduced into the recipient subject. (Page 37, lines 20-25).

The use of viral vectors suffers from their potential for rearrangement of endogenous genes, as well as their potential for inducing carcinogenesis. (Page 6, lines 28-31).

In the past few years, it has become apparent that the implementation of retroviral based gene delivery systems will face major obstacles, primarily related to properties of retroviruses themselves (Robertson, M. Nature 320:213-214 (1986), Marx, J.L., Science 232:824-825 (1986)). (Page 4, lines 16-21).

First, it has not been generally possible to achieve expression of mammalian genes in the retroviral vectors used to infect human cells, and until this problem is solved, the issue of regulated gene expression cannot be addressed. (Id., lines 21-25).

Second, when retroviruses are used to infect marrow cells in batch essentially every cell is infected, and the site of retroviral integration into the host genome varies from cell to cell. Since the infected cells are not characterized before introduction, the possibility of a deleterious integration event cannot be eliminated. (Id., lines 25-31).

LAW OFFICES

FINNEGAN, HENDERSON,
FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N.W.
WASHINGTON, DC 20005
202-408-4000

wherein the DNA sequence comprises the gene and a promoter operably linked to the gene;

Third, as recombination between the replication-deficient retroviruses utilized for infection and the endogenous retroviruses present in mammalian genomes is known to occur (Hock, R.A., et al., Nature 320:275-277 (1986)), there is the potential of initiating a chronic retroviral infection in the host animal. (Page 5, line 32 - page 5, line 4).

In order to accomplish gene expression, it is necessary that the structural sequences of the desired of [sic, or] effector gene be operably linked to a promoter. (Page 18, lines 28-31).

A gene sequence is to be operably linked to a promoter region if the linkage is sufficient to enable the gene sequences to be transcribed due to the presence of the promoter region. In one embodiment of the present invention, the desired or effector DNA sequences are introduced into the transfected cell linked to their normal and natural promoter regions. Such transfected cells are capable of producing the product of the desired or effector gene under that gene's normal regulatory control. Alternatively, in a preferred embodiment, it is possible to link a particular desired or effector gene sequence to a promoter region with which it is not normally associated. Thus, any promoter capable of functioning in the transfected cell can be operably linked to the desired or effector gene sequence and used to express the gene in the transkaryotic cell. (Page 18, line 33, through page 19, line 16).

(b) screening the resulting transfected somatic cells *in vitro* to select a cell

Stably transfected cells are identified by their ability to express the marker gene

possessing desired expression properties;

as evidenced by their ability to survive the selection regimen (cells that have not taken up the marker gene are destroyed by this regimen). (Page 12, lines 26-30).

Individual colonies of stably transfected cells are then multiplied and characterized with respect to the expression and regulation of the gene of therapeutic interest. A clonal line possessing the desired expression properties (Page 12, line 30 - page 13, line 3).

Add Selection. (Fig. 1).

In order to identify stably transfected cells, it is, in general, necessary to screen or select such cells from the total culture of transfected cells. Thus, it is desirable to co-transfect the transfected cell with a second gene sequence capable of conferring a selectable property to the cell A transfected cell which exhibits a stable expression of a selectable property is examined to determine whether it also expresses the desired gene sequences. (Page 35, line 15 - page 36, line 2).

(c) cloning and expanding the selected somatic cell *in vitro*; and

Individual colonies of stably transfected cells are then multiplied and characterized with respect to the expression and regulation of the gene of therapeutic interest. A clonal line possessing the desired expression properties (Page 12, line 30 - page 13, line 6).

Clone Individual Colony. (Fig. 1).

After transfection, it is preferable to allow the transfected cells to proliferate (Page 36, lines 26-29).

(d) administering the resulting cloned and expanded somatic cells to the animal.

A clonal cell line possessing the desired expression properties is then introduced into one of a variety of anatomical locations in the host animal (Page 13, lines 2-6).

Introduce Cells Into Desired Anatomical Location. (Figure 1).

Independent claim 87 is identical to independent claim 72 except that it recites "without a retroviral vector" rather than "without a viral vector." The specification supports this recitation at, *inter alia*, page 4, line 16, through page 5, line 4:

In the past few years, it has become apparent that the implementation of retroviral based gene delivery systems in humans will face major obstacles, primarily related to properties of retroviruses themselves (Robertson, M., Nature 320:213-214 (1986), Marx, J.L., Science 232:824-825 (1986)). First, it has not been generally possible to achieve expression of mammalian genes in the retroviral vectors used to infect human cells, and until this problem is solved, the issue of regulated gene expression cannot be addressed. Second, when retroviruses are used to infect marrow cells in batch, essentially every cell is infected, and the site of retroviral integration into the host's genome varies from cell to cell. Since the infected cells are not characterized before reintroduction, the possibility of a deleterious integration event cannot be eliminated. Third, as recombination between the replication-deficient retroviruses utilized for the infection and the endogenous retroviruses present in mammalian genomes is known to occur (Hock, R.A., *et al.*, Nature 320:275-277 (1986)), there is the potential of initiating a chronic retroviral infection in the host animal.

The specification and originally filed claims also support the new claims that depend from claims 72 and 87. These new dependent claims contain recitations that Applicant included in the dependent claims that Applicant previously submitted during the prosecution of this application. (See, e.g., specification at 62, line 1, through 65, line 4).

Because the original claims did not recite "does not involve a viral vector," nor did they recite "does not involve a retroviral vector" or "comprises no DNA of retroviral origin," Applicant will further discuss the specification's support for these recitations.

The written description requirement is directed to a "person skilled in the art." 35 U.S.C. § 112, first paragraph. Accordingly, the disclosure as filed must only convey to one skilled in the art that Applicant invented the claimed subject matter. Vas-Cath, Inc. v. Mahurkar, 19 U.S.P.Q.2d 1111, 1117 (Fed. Cir. 1991). Applicant respectfully submits that the specification provides an adequate written description for the recitation of "does not involve a viral vector," "does not involve a retroviral vector," and "comprises no DNA of retroviral origin" because the specification would have conveyed to one skilled in the art that Applicant had invented the claimed method, which does not involve a viral or retroviral vector and which involves no DNA of retroviral origin.

The specification and original claims continually use the terms "transfect," "transfection," and "transfected." Transfection is distinct from transduction, which involves infection with a viral vector. The viral vector infects a cell and introduces genes into the cell. Unlike infection, transfection does not involve a viral vector. Transfection involves using physical and chemical techniques to introduce a gene into a cell without

a viral vector. Indeed, every article the specification cites as teaching transfection methods does not involve using a viral vector to infect cells and deliver a gene. (See specification at 36, lines 7-13).

Applicant used nonviral transfection rather than viral infection because of the numerous problems with "retroviral based gene delivery systems," which, as Applicant showed in the above chart, the specification discusses in detail. (Id. at 4, line 16, through 5, line 4). These very problems are related to the "properties of retroviruses themselves." (Id. at 4, lines 16-19). It is not surprising, therefore, that at least three of these problems pertain to the use of any DNA of retroviral origin, not just the use of viral or retroviral vectors.

As can be seen in Fig. 1 of the Anderson reference the Examiner cited, a retroviral genome has two parts: 1) the regulatory elements ("LTR"), including a promoter; and 2) the retroviral structural genes themselves. These elements of retroviral nucleic acid are the source of the problems with retroviral based gene delivery systems that Applicant discusses in the specification.

For example, the specification states:

First, it has not been generally possible to achieve expression of mammalian genes in the retroviral vectors used to infect human cells, and until this problem is solved, the issue of regulated gene expression cannot be addressed.

(Specification at 4, lines 21-25). In other words, expression of the desired gene had not generally been achieved with retroviral vectors. Retroviral vectors use the retroviral LTRs, which control the expression of retroviral genes. (Anderson at 406, col. 1).

Accordingly, a problem with retroviral promoters for gene delivery is that they result in little, if any, expression of the gene of interest.

The specification also states:

Second, when retroviruses are used to infect marrow cells in batch essentially every cell is infected, and the site of retroviral integration into the host genome varies from cell to cell. Since the infected cells are not characterized before introduction, the possibility of a deleterious integration event cannot be eliminated.

(Id. at lines 25-31). In other words, since DNA of retroviral origin can integrate anywhere in a host cell's genome, it can disrupt an important gene or regulatory sequence of the host. (Anderson at 407, col. 2). Indeed, this is the very concern of the Robertson article the specification cites regarding the difficulties of retroviral based gene delivery systems being due to the properties of retroviruses themselves.

(Robertson, Miranda, Desperate Appliances, 320 Nature 213, 214, col. 1 (1986) (copy enclosed)).

The specification next states the most important problem with DNA of retroviral origin in gene delivery systems:

Third, as recombination between the replication-deficient retroviruses utilized for infection and the endogenous retroviruses present in mammalian genomes is known to occur (Hock, R.A., et al., Nature 320:275-277 (1986)), there is the potential of initiating a chronic retroviral infection in the host animal.

(Specification at 4, line 32, through 5, line 4).

All animals have retroviral sequences ("endogenous retroviruses") integrated into their genomes. These proviral sequences are often harmless. However, their

activation has been implicated in some cancers. Since exogenous DNA of retroviral origin can recombine with the nucleic acid of the endogenous retroviruses, it can activate the endogenous nucleic acid, perhaps resulting in a cancer cell. Furthermore, the recombination of the exogenous retroviral nucleic acid with the endogenous retroviral nucleic acid can create a new retrovirus capable of infecting the host. (Anderson at 407, col. 1-2).

Applicant also summarized the problems with viral vectors:

At present, however, no single technique appears to be wholly satisfactory. The use of viral vectors suffers from their potential for rearrangement of endogenous genes, as well as their potential for inducing carcinogenesis.

(Specification at 6, lines 27-31).

Given the specification's discussion of these three disadvantages and dangers of viral and retroviral vector gene delivery systems and DNA of retroviral origin, the specification then goes on to describe a solution to these problems, which does not involve the use of viral vectors, retroviral vectors, or DNA of retroviral origin. Specifically, the specification describes transfecting somatic cells without a viral vector and with no DNA of retroviral origin; screening the transfected somatic cells; cloning and expanding a selected somatic cell; and administering the resulting cloned and expanded cells to the recipient subject. (See, e.g., id. at 12, line 22, through 13, line 6). By not using a viral vector, by not using DNA of retroviral origin, by selecting the transfected cells, and by cloning and expanding the selected cells, the claimed invention avoids these three disadvantages and dangers of viral vectors, retroviral vectors, and DNA of retroviral origin.

Applicant's invention is further evident from the discussion of particular promoters at pages 18-19. One reason many in the field focused upon viral vectors was because their own regulatory elements could be used easily. (Anderson at 406, col. 1). In the more than one page discussing particular promoters, Applicant's specification does not discuss a single viral or retroviral promoter. Rather, Applicant discusses promoters from mammals, fruit flies, and yeast. This is striking because viral and retroviral promoters were the promoters being used by those skilled in the art. The absence of any discussion of viral or retroviral promoters, combined with the discussion of the disadvantages and dangers of viral vectors, retroviral vectors, and DNA of retroviral origin, would have conveyed to one skilled in the art that, in one embodiment, Applicant invented a method of transferring a gene into a recipient subject that does not involve a viral vector, a retroviral vector, or DNA of retroviral origin.

This is **all** the written description requirement of § 112, first paragraph, requires: that the disclosure convey to one skilled in the art that Applicant invented the claimed subject matter. Vas-Cath, 19 U.S.P.Q.2d at 1117. In other words, the disclosure need only indicate to one skilled in the art that Applicant had possession of the claimed subject matter. Ralston-Purina Co. v. Far-Mar Co., Inc., 227 U.S.P.Q. 177, 179 (Fed. Cir. 1985). Since the written description requirement is directed to a person skilled in the art, it "does not require that the claimed invention be described in the same words, i.e., *ipsis verbis*." Ex parte Yamaguchi, 6 U.S.P.Q.2d 1805, 1807 (PTO Bd. Pat. App. & Int. 1988); see also M.P.E.P. § 2063.03) ("the subject matter of the claim need not be described literally (i.e., using the same terms or in *haec verba*, in order for the

disclosure to satisfy the description requirement"). The specification's discussion of the serious problems with viral vectors, retroviral vectors, and DNA of retroviral origin in gene delivery systems, the absence of any other discussion of viral vectors, retroviral vectors, and DNA of retroviral origin, and the specification's use of neither viral vectors, retroviral vectors, nor DNA of retroviral origin in the examples would have indicated to one skilled in the art that Applicant had possession of a method of transferring a gene that does not involve a viral vector, a retroviral vector, or DNA of retroviral origin.

Background of the Invention

Applicant will summarize the background of the invention because the Examiner appears to have confused Applicant's nonviral gene transfer and gene therapy work with viral, especially retroviral, gene transfer and gene therapy, and because the Examiner appears to have assumed that the failures of viral methods apply to Applicant's nonviral methods. Applicant's nonviral gene transfer and gene therapy are very different from viral gene transfer and gene therapy, and the failures of the viral methods do not apply to Applicant's nonviral methods. Indeed, the repeated failures of the viral methods stand in stark contrast to the successes of Applicant's nonviral methods.

Applicant published two articles in 1987 that report some of the results contained in this application (Selden et al., 236 *Science* 714-718 (1987) (copy enclosed) and Selden, R., 317 *N. Eng. J. Med.* 1067-1076 (1987) (copy enclosed). Applicant subsequently became associated with Transkaryotic Therapies, Inc. ("TKT"). TKT was founded based upon the work from Applicant's two 1987 articles.

LAW OFFICES

FINNEGAN, HENDERSON,
FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N.W.
WASHINGTON, DC 20005
202-408-4000

Several other gene therapy companies were founded around the time that TKT was founded, including Viagene, Somatix, and Genetic Therapies, Inc. ("GTI"). TKT is the only gene therapy company, however, to use nonviral transfection as its primary gene delivery system for *ex vivo* gene transfer and gene therapy. Every single other gene therapy company has channeled its efforts into viral infection ("transduction"), especially retroviral infection, for *ex vivo* gene transfer and gene therapy.

None of these other gene therapy companies continues to exist as an independent company today. TKT, in contrast, continues to exist as an independent company, making it the oldest surviving gene therapy company.

But TKT is not just existing; it is thriving. TKT completed a highly successful Initial Public Offering in late 1996. TKT also continues to attract significant investments from both individuals purchasing its stock and from corporations licensing TKT's technology.

As a small company highly dependent upon its proprietary technologies, TKT has always had rigorous proprietary policies. As a result of these policies, TKT's employees seldom publish the results of their research in journals. Rather, they publish their results in the form of patent documents after TKT has filed a patent application. Applicant, Dr. Richard F Selden, became subject to these proprietary policies upon becoming associated with TKT. Therefore, the work contained in this application that was not published in Applicant's two 1987 articles has not been published in the U.S., and it will not be published in the U.S. until a patent issues from this application.

Since TKT is the only gene therapy company focusing on nonviral gene transfer and gene therapy, and since TKT seldom publishes its work in journals, the Examiner's confusion of Applicant's nonviral gene transfer and gene therapy with viral gene transfer and gene therapy and the failures of these viral methods is understandable. Nonetheless, Applicant's nonviral methods are quite distinct from the viral methods, and the failures of the viral methods do not apply to Applicant's nonviral methods. Indeed, while viral methods continue to fail, Applicant's nonviral methods continue to succeed. Other researchers continue to build upon the portion of Applicant's work published in 1987, and they recognize this work as pioneering. These researchers credit Applicant's work with opening a whole new field--the field of nonviral gene transfer and gene therapy.

Rejections of Claims 69-71 Under 35 U.S.C. § 101

The Examiner rejected claims 69-71 under 35 U.S.C. § 101 as allegedly being directed to nonstatutory subject matter. Applicant fails to understand how these claims, which recite implants, were directed to nonstatutory subject matter, and the Examiner's explanation is not instructive because it concerns claims 33-35, which Applicant canceled in the October 29, 1996, Amendment. In any case, solely to expedite prosecution of this application, and not in acquiescence to this rejection, Applicant has canceled claims 69-71, so this rejection is moot.

Provisional Double Patenting Rejections

The Examiner provisionally rejected claims 37-71 under the judicially created doctrine of obviousness type double patenting over: (1) claims 34-71 in copending Application Serial No. 08/460,902; (2) claims 135-161 of copending Application Serial No. 08/334,797; (3) claims 108-132 of copending Application Serial No. 08/334,455; (4) claims 108-132 of copending Application Serial No. 08/451,894; (5) claims 68-77 and 105-107 of copending Application Serial No. 08/446,909; (6) claims 125-134 of copending Application Serial No. 08/446,912; and (7) claims 125-134 of copending Application Serial No. 08/443,936. (Paper No. 10 at 3-6). Applicant respectfully traverses these provisional rejections and will deal with them on the merits when the subject application is found to contain allowable subject matter.

Written Description Rejection of Claims 37-71 under 35 U.S.C. § 112, First Paragraph

The Examiner rejected claims 37-71 under 35 U.S.C. § 112, first paragraph, as allegedly not being supported by an adequate written description. Applicant respectfully traverses this rejection.

As Applicant discussed above, the written description requirement merely requires that the specification indicate to one skilled in the art that Applicant invented the claimed subject matter. Vas-Cath, 19 U.S.P.Q.2d at 1117. Applicant respectfully submits that the specification would have indicated to one skilled in the art that Applicant invented the claimed method for use with all animals and any genes, despite the Examiner's apparent contention to the contrary.

The specification broadly refers to "recipient subject" throughout. It also expressly states that the "subject recipient" includes animals in general and humans in particular. (Specification at 15, lines 18-22). Similarly, the specification refers broadly to "gene product" throughout, including the very first sentence of the Summary of the Invention. (Specification at 9). Furthermore, the specification expressly discusses genes for factor VIII (id. at 2); neomycin phosphotransferase, adenosine deaminase, hypoxanthine phosphoribosyltransferase (id. at 4); dihydrofolate reductase (id. at 5); tissue plasminogen activator (id. at 20); somatostatin (id. at 22); antibodies, cellular receptor molecules, hormones, and enzymes (id. at 25); and thymidine kinase (id. at 35). The specification also states that the invention involves "the use of any transfected cell capable of expressing **any gene** in a recipient subject." (Id. at 39, lines 2-4; emphasis added).

Finally, the specification also states:

It is to be understood that the present invention is not limited to the use of such transfected cells, but rather encompasses the use of any transfected cell capable of expressing any gene in a recipient subject. Examples of other genes which could alternatively have been employed include genes for hormones, enzymes, antibodies, and the like.

(Specification at 38-39).

From these passages, as well as the specification as a whole, one skilled in the art would have recognized that Applicant invented the claimed subject matter for all animals and for any genes. Accordingly, Applicant respectfully requests withdrawal of this rejection.

Enablement Rejection of Claims 37-71 under 35 U.S.C. § 112, First Paragraph

The Examiner rejected claims 37-71 under 35 U.S.C. § 112, first paragraph, as allegedly not being enabled by the specification on several bases. Applicant respectfully traverses this rejection.

I. The pending claims expressly recite gene transfer, not gene therapy.

Ex vivo gene therapy involves three basic steps: (1) the introduction of foreign genetic material into cells *in vitro*, followed by the transfer of cells containing the inserted genetic material into a patient ("gene transfer"); (2) expression of the foreign genetic material in the patient ("*in vivo* expression"); and (3) expression at levels sufficient to produce a therapeutic effect, for example, so as to correct a disease state or disorder related to the inability of the patient to make the protein encoded by the foreign genetic material ("therapeutic effectiveness"). Gene therapy, thus, requires the *in vivo* expression of a gene at a level that will have a therapeutic effect.

A review of Applicant's claims will show that Applicant is claiming a method of transferring a gene into a recipient subject. The method involves transfecting somatic cells, screening the transfected somatic cells; cloning and expanding a selected somatic cell; and administering the resulting cloned and expanded somatic transfected cells to the recipient subject. Before gene therapy can become a common procedure, methods for transferring genes into animals are needed, and the claimed gene transfer method constitutes a useful system for transferring a gene into an animal.

To the extent that the rejection concerns gene therapy, including all three of its component steps, Applicant respectfully submits that it is irrelevant to the claimed

invention. Applicant will, nonetheless, address each of these grounds for rejection in case the Examiner contends that any of them apply to gene transfer, the first of gene therapy's three component steps. Applicant expressly reserves the right to pursue claims to *ex vivo* gene therapy in this or another application.

II. The pending claims do not concern plants.

The Examiner rejected the claims as not being enabled for all "recipient subjects" because this term can allegedly also include plants. As Applicant showed above, the specification defines "recipient subject" as including animals; it does not define "recipient subject" as including plants. Solely to expedite prosecution of the pending claims, Applicant's new claims specifically recite that the recipient subject is an animal. Thus, this ground for rejection is moot.

III. Applicant demonstrated gene transfer in an established animal model of a human disease.

The Examiner also based the enablement rejection upon the alleged inability of mouse examples to predict results in humans. This basis for the enablement rejection fails because Applicant proved gene transfer in an established model of human disease, namely, a rodent model of diabetes, by proving therapeutic effectiveness.

"[A] specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented **must** be taken as in compliance with the enablement requirement of the first paragraph of § 112 unless there is reason to doubt the objective truth of the statements contained therein

which must be relied on for enabling support." In re Marzocchi, 169 U.S.P.Q. 367, 369 (C.C.P.A. 1971) (emphasis added). As Applicant explained above in response to the apparent written description rejection, the specification contains enabling teachings commensurate in scope with the claims.

For example, although the Examples use mice, the specification states that the "subject recipient" into which the desired gene is introduced includes all animals. (Specification at 15, lines 18-22). Similarly, although the Examples use fibroblasts, the specification indicates that the invention can also be practiced with "myocytes, hepatocytes, kidney capsular cells, endothelial cells, epithelial cells of the gut, pituitary cells, etc." (Id. at 37, lines 25-28). Although the Examples use the human growth hormone and insulin genes, the specification states that the invention can be used with genes for all "hormones, enzymes, antibodies, and the like." (Id. at 39, lines 4-5). Finally, the specification even states that the invention "encompasses the use of any transfected cell capable of expressing any gene in a recipient subject." (Id. at lines 2-4). Since the specification contains enabling teachings commensurate in scope with the claims, it must be taken as in compliance with the enablement requirement of the first paragraph of § 112 unless the Examiner provides reason to doubt the objective truth of these statements.

When the Examiner believes there is reason to doubt the objective truth of Applicant's statements, he must support it with evidence or reasoning. Marzocchi, 169 U.S.P.Q. at 370. The only evidence or reasoning the Examiner provides is a general statement that results in mice do not always predict what will happen in humans

and a statement regarding the failure of others to obtain expression of a protein in mice. Applicant respectfully submits that this is not evidence or reasoning sufficient to doubt the objective truth of the statements in the specification, especially since the present claims concern gene transfer, and the Examiner's evidence concerns, not gene transfer, but *in vivo* expression and therapeutic effectiveness. Nonetheless, Applicant will provide further evidence that the specification is enabling.

The use of standard experimental models has often been recognized as supporting broader claims for purposes of 35 U.S.C. § 112, first paragraph. See, e.g., In re Jolles, 206 U.S.P.Q. 885, 890 (C.C.P.A. 1980); Application of Hartop, 135 U.S.P.Q. 419, 426 (C.C.P.A. 1962).¹ Applicant's demonstration that the selected and cloned cells of the invention had desirable and beneficial properties in the alleviation of diabetes in an experimental animal satisfies § 112, first paragraph. As the court pointed out in Jolles: "this court has accepted tests on experimental animals as sufficient . . . because of the widespread pharmacological work in animals recognized as a screening procedure for testing new drugs." Jolles, 206 U.S.P.Q. at 890.

Example 10 of the specification demonstrates the use of transkaryotic implantation to treat a human disease (diabetes) in an established animal model of the human disease:

¹ Although these cases concern utility, not enablement, their logic applies equally to enablement. Both requirements appear in the same paragraph of § 112, and both requirements use the same time frame (the filing date) and the same reference (one skilled in the art). Furthermore, the close relationship between utility and enablement has been recognized by the PTO, see, e.g., Utility Examination Guidelines, 60 Fed. Reg. 36263 (July 14, 1995); Legal Analysis Supporting Utility Examination Guidelines, and by the Federal Circuit. See, e.g., In re Brana, 34 U.S.P.Q.2d 1436, 1439 (Fed. Cir. 1995); Raytheon Co. v. Roper Corp., 220 U.S.P.Q. 592, 596 (Fed. Cir. 1983).

In Example 10, the treatment of diabetes was modeled by monitoring the effect of intraperitoneally injected Ltk⁺Ins cells on diabetic mice. To obtain chemically diabetic animals, C3H mice were treated with streptozotocin, and serum glucose levels were measured approximately 10 and 15 days later.

* * *

Within one week post-implantation, a dramatic decline in serum glucose levels was noted in five of the mice (Figure 9, solid line). By two weeks post-implantation, normoglycemia was restored in these diabetic mice.

(Specification at 52, lines 5-21).

Streptozotocin-induced diabetes in the mouse had been used for a number of years before Applicant's invention. Like and Rossini described it as early as 1976. (Like et al., Science 193:415 (1976) (copy enclosed)). Stearns et al. commented that "animals injected with the antibiotic streptozotocin (SZ) have an acute insulin deficit resembling that of individuals with insulin-dependent diabetes mellitus." (Stearns et al., Acta Anat. 15:193-203 (1983), at 194, col. 1 (copy enclosed)). In 1986, shortly before Applicant's filing date, Schwab commented further on the streptozotocin diabetes model stating that: "Low dose streptozotocin-induced diabetes serves as a model of human-type I diabetes," referring to the Like and Rossini 1976 article. (Schwab et al., Immunopharmacology 12:17-21 (1986), at 17, col. 1 (copy enclosed)).

Applicant's diabetes expert, Karl Geisen, M.D., had the following additional comments on the value and relevance of the streptozotocin-induced diabetes model in human diabetes therapy. (Declaration of Karl Geisen (copy enclosed)).

The use of animals rather than humans in diabetes research has several advantages. Animal models provide an opportunity for investigating the effects of therapeutic agents developed for the prevention, treatment, or cure of

LAW OFFICES

FINNEGAN, HENDERSON,
FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000

diabetes and its complications before the therapeutics are administered to humans. Well-studied animal models have permitted experienced researchers to correlate between animal and human diabetes.

A diabetes-like condition can be induced in rodents by pharmacologic intervention. More particularly, a well characterized model of Type I diabetes can be induced by administering the chemical agent streptozotocin to mammals, such as mice, rats, rabbits, dogs, and pigs.

(Id. at ¶ 14-15).

The destruction of β -cells and hyperglycemia are caused by streptozotocin treatment. The streptozotocin-treated mouse model has been known and used in the study of Type I diabetes for over 20 years. β -cell destruction is the primary defect in this streptozotocin model of diabetes disease. Normal blood glucose levels in the streptozotocin model can be restored by insulin treatment. Thus, use of the rodent model provides a means for studying experimental diabetes. (Id. at ¶¶ 16-18).

It has been Dr. Geisen's experience that an insulin therapy that was found to be effective in the treatment of streptozotocin-induced diabetes in a mouse model would also exhibit effectiveness in the human. In Dr. Geisen's opinion, the insulin therapy, which Applicant showed was effective in the streptozotocin-induced diabetic C3H mouse model, would be expected to be effective in humans. (Id. at ¶¶ 21-22).

Dr. Geisen's opinion is based on his experience, which has included evaluating diabetes therapies in streptozotocin models of diabetes, the extension of these therapies to clinical trials in humans after effectiveness was shown in the streptozotocin model, and correlation of the results in the animal model with the results in humans for the same insulin therapy. It has been his experience that there is very high correlation

between the results of a given insulin therapy in streptozotocin-induced diabetes in a mouse model and the results of the same insulin therapy in clinical trials in humans. (id. at ¶ 23).

In summary, Applicant's use of the streptozotocin-induced model as a predictor of success in humans was consistent with the use of this model for humans for a period of more than 10 years before Applicant's invention. Thus, Applicant demonstrated gene transfer and, indeed, gene therapy, in an experimental animal model that was predictive of results in humans.

IV. Immunosuppressive therapy is merely an optional embodiment.

The Examiner also based the enablement rejection on Applicant's use of immunosuppression in some of the Examples. The use of immunosuppression does not support the Examiner's enablement rejection because immunosuppression is merely an alternative embodiment of the gene transfer and gene therapy method described in the specification. Applicant's invention can be used to both permanently and transiently express a desired gene. (See, e.g., specification at 33-34; discussing "stably transfected" and "transiently transfected" cells). Gene transfer need not be used solely for therapy where the desired gene will be permanently expressed. It can also be used for "various temporary interventions" where the desired gene need only be expressed for a short period of time. (See id. at 20, lines 10-24). If further protein is desired, the gene transfer can be repeated.

Furthermore, as Applicant discussed in the specification (id. at 38, lines 1-9), and as the Examiner recognizes (Paper No. 10 at 12, lines 16-17), one skilled in the art

would have known to use cells that are antigenically similar to the recipient subject, preferably, autologous cells, to minimize the expected immune response to the administered cells. Applicant even observed in the specification that it is likely that, although the Ltk⁺ cells were originally obtained from C3H mice, four decades of genetic drift in the cells and in the mice resulted in the cells and mice no longer being syngeneic. (Specification at 46, line 26, through 47, line 1). Applicant also concluded that the introduced cells died because the host's immune system attacked them. (Id. at 47, lines 9-12).

Applicant anticipated that some patients may need a therapy to supplement the somatic cell gene transfer, for example, to prolong the functional life of a cell implant, so that the gene transfer need not be repeated. Applicant proposed a regimen of immunosuppression. (Specification at 38, lines 9-19). Implementation of an immunosuppressive regimen is specifically exemplified in the specification in Examples 6-7 on pages 46-49.

Others have used immunosuppression to enhance the efficacy of gene transfer, just as immunosuppression is widely used in the treatment of patients with organ transplantation and multiple sclerosis. For example, in "Treatment of human hepatocellular carcinoma by fibroblast-mediated human interferon α gene therapy in combination with adoptive chemo-immunotherapy", Cao et al used an immunotherapy in combination with somatic cell gene therapy. (Cao et al., Cancer Res. Clin. Oncol. 121:457-462 (1995) (copy enclosed)).

Immunosuppression of the patient has advantages as other workers in this field have recognized. In any event, Applicant's use of the word "alternatively" in describing the use of an immunosuppressive drug leaves no doubt that immunosuppression of the patient is an optional embodiment of the invention. Indeed, Examples 2-5 in the specification were conducted without immunosuppression of the subject.

**V. The specification is enabling for genes and animals
not used in the examples.**

The Examiner also asserts that the specification does not enable a composition for use with animals other than mice and genes other than the insulin gene. Applicant respectfully traverses this rejection.

**A. The specification demonstrates
gene transfer for two genes.**

The claims recite a method of transferring a gene. Examples 2-8 teach such a method where the gene transfer of the human growth hormone gene is proved by the altered concentration of human growth hormone *in vivo*. Examples 9 and 10 teach a composition for transferring a gene where the gene transfer of the insulin gene is proved by the altered concentration of insulin.

These nine examples of successful nonviral gene transfer with two different genes stand in contrast to the absence of any evidence of any failures of *ex vivo* nonviral gene transfer. These successes, therefore, are sufficient evidence of the enablement of the claimed invention for other genes and for other animals. Cf. *Ex parte Balzarini*, 21 U.S.P.Q.2d 1892, 1897 and n.7 (Bd. Pat. App. & Int. 1991)

(Despite evidence of previous failures, data from testing in standard experimental animals may establish utility and enablement.).

**B. The Orkin et al. and Crystal references did not
consider Applicant's category of gene transfer.**

Despite this evidence, the Examiner relies upon the Orkin et al. and Crystal references as allegedly supporting the rejection. Applicant respectfully submits that these references are irrelevant to the enablement of the claimed invention because they did not consider Applicant's category of gene transfer--*ex vivo* nonviral gene transfer.

The field of gene transfer can be divided into four basic, distinct categories:

<i>ex vivo</i> viral	<i>in vivo</i> viral
<i>ex vivo</i> nonviral	<i>in vivo</i> nonviral

In its report on the status of the field of gene therapy, the Orkin et al. reference only discusses three of the four distinct gene transfer methods (*ex vivo* viral gene transfer; *in vivo* viral gene transfer; and *in vivo* nonviral gene transfer). (Orkin et al. at 7-9). It does not discuss any *ex vivo* nonviral gene transfer methods. Applicant's gene transfer method is intended for *ex vivo* use and is nonviral. Accordingly, the Orkin et al. reference is irrelevant to the claimed invention.

The Crystal reference is also irrelevant because it too does not consider *ex vivo* nonviral gene transfer. Like the Orkin et al. reference, the Crystal reference discusses three of the four categories of gene transfer (*ex vivo* viral; *in vivo* viral; and *in vivo*

nonviral). (Crystal at 404-408). It does not discuss any *ex vivo* nonviral gene transfer methods. Because the Orkin et al. and Crystal references do not consider *ex vivo* nonviral gene transfer, they are irrelevant to the claimed invention and do not constitute evidence supporting the enablement rejection.

**C. The Orkin et al. and Crystal references
did not consider Applicant's work.**

The Orkin et al. and Crystal references could not have considered Applicant's work because, as Applicant shows below, they only considered gene transfer work that the National Institutes of Health ("NIH") funded. The Recombinant DNA Advisory Committee ("RAC") of NIH oversaw and approved all gene transfer work funded by the NIH. All the proceedings of the RAC are publicly available.

The Orkin et al. reference is the Report and Recommendations of the Panel to Assess the NIH Investment in Research on Gene Therapy. As the title of this panel indicates, it was charged with evaluating the NIH's investment in gene therapy:

Dr. Harold Varmus, Director, National Institutes of Health (NIH), appointed an *ad hoc* committee to assess the current status and promise of gene therapy and provide recommendations regarding future NIH-sponsored research in this area. The Panel was asked specifically to comment on how funds and efforts should be distributed among various research areas and what funding mechanisms would be most effective in meeting research goals.

(Orkin et al. at 1).

Given this mandate, the panel focused on RAC-approved gene therapy protocols. For example, the purpose of the first meeting of the Panel was "to provide the Panel with an opportunity to hear presentations regarding the current and

anticipated research activities relevant to gene therapy **that are supported by various components of NIH**, and to discuss how to proceed with its assessment of NIH's investment in gene therapy." (Notice of Meeting of the Panel to Assess the NIH Investment in Research on Gene Therapy, 60 Fed. Reg. 20737 (1995) (emphasis added) (copy enclosed)).

Similarly, the Panel's final report indicates it focussed on RAC-approved protocols. The "Gene therapy in man status of the field" section indicates that the Panel based its conclusion that clinical efficacy has not been demonstrated, the conclusion upon which the Examiner relies, on a review of only RAC-approved gene therapy protocols. "Upon reviewing the status of clinical protocols approved for gene transfer, the Panel made several observations: Efficacy has not been established for any gene therapy protocols." (Orkin et al. at 13, lines 14-16). Accordingly, the Orkin et al. reference is specifically limited to those protocols submitted to and approved by the RAC.

The Crystal reference the Examiner relies upon similarly focussed on protocols the RAC approved: "Human data in this review are derived from published articles and abstracts and from the December 1994 and June 1995 RAC investigator reports." (Crystal at 410 n.4). Indeed, Crystal believes that the RAC reports "are an accurate gauge of the status of the field" (Id.). Although Crystal states that he considered published articles and abstracts, these articles and abstracts consist solely of those resulting from RAC-approved gene transfer protocols.

The Orkin et al. and Crystal references did not address Applicant's work because it was not considered by the RAC. Applicant's studies in gene transfer and gene therapy were privately funded. They were not funded by the NIH. Since Applicant's studies were not funded by the NIH, they did not required to be disclosed to the RAC or approved by the RAC.

As Applicant discussed above, Applicant became associated with TKT after publishing two articles in 1987 that contain some of the results contained in this application. Unlike the scientists who had no choice but to seek RAC approval because of NIH funding, TKT chose the more conventional path for the oversight of pharmaceuticals in development, which was to seek approval of the Food and Drug Administration ("FDA"). The FDA is, of course, vested with authority to review and approve the use of therapeutic agents in humans.

Furthermore, compliance with the enablement requirement "must be judged as of the filing date." In re Glass, 181 U.S.P.Q. 31, 34 (C.C.P.A. 1974). Thus, references illustrating the state of the art after an application's filing date cannot be used to render the claims not enabled. U.S. Steel v. Philips Petroleum Co., 9 U.S.P.Q.2d 1461, 1465 (Fed. Cir. 1989). Thus, the Examiner cannot use a later reference to allegedly show that Applicant's claimed invention is not enabled. This is especially true in the case of the Orkin et al. and Crystal references, which do not discuss any of Applicant's work, nor even any *ex vivo* nonviral gene transfer methods, either before or after Applicant's effective filing date.

In contrast to the Orkin et al. and Crystal references, which the Examiner relies upon to support the enablement rejection, references that actually considered Applicant's work have recognized it as novel and pioneering. As a pioneering invention, Applicant is entitled to broad claims to the broad concept Applicant enabled. In re Hogan, 194 U.S.P.Q. 527, 537 (C.C.P.A. 1977). If later references could be used to allegedly show that the claimed invention is not enabled, "the opportunity for obtaining a basic patent upon early disclosure of pioneer inventions would be abolished." Id.

**D. Others Have Recognized Applicant's
Novel And Pioneering Work.**

Kawakami et al. recognized Applicant's work as pioneering in the field of somatic gene therapy:

Somatic gene therapy has been performed in an animal model of diabetes and also has been used as a growth hormone-supplying system in mice (2-4,5). These efforts pioneered the field of somatic gene therapy for hormone deficiency by proving that even a few fibroblasts and keratinocytes can supply enough hormone to these animal models.

(Kawakami et al. (Diabetes 41:956-961(1992)) at 956 (copy enclosed)). Reference "2" is a citation to Applicant's 1987 *Science* article, and reference "4" is a citation to the 1987 *New England Journal of Medicine* article.

Even as recently as 1994, Simpson et al. paid tribute to the pioneering nature of Applicant's work:

Experiments with mice bearing an intact human insulin gene inserted into mouse Ltk fibroblast cells pioneered the field of somatic gene therapy in diabetes by proving that transfected fibroblasts can supply enough insulin in diabetic mice to normalize their blood glucose.²

(Simpson et al., (Gene Therapy 2:223-231 (1995) (copy enclosed)), at 223). Footnote "2" is a citation to Applicant's 1987 *New England Journal Of Medicine* article.

Recently, Lauffenburger et al. referred to Applicant's transkaryotic implantation method as a "primary example" of a method for transporting growth factors through cellular matrices. (Lauffenburger et al., (Biotechnology and Bioengineering 52: 61-80 (1996) (copy enclosed)), at 71, col. 1).

These reports pay tribute to a technique that was rejected by the gene therapy researchers who focused on viral gene transfer methods. Applicant's ground-breaking efforts opened the field of *ex vivo* nonviral gene therapy to others. But TKT is not the only entity building on Applicant's early discoveries.

**E. Applicant's Early Discoveries
Continue To Be Utilized Outside TKT**

By 1988, shortly after the subject application was filed in the PTO, Rosenberg et al. acknowledged Applicant's contribution to the field of human gene therapy. (Rosenberg et al., Reports, pp. 1575-1578 (1988) (copy enclosed)). Commenting on Applicant's published gene therapy method in their article in the journal *Science*, Rosenberg et al. stated that their "present study . . . extends the feasibility of such an approach", a clear statement that they endorsed Applicant's discovery. *Id.* at 1576.

By 1989, Tani et al. showed that Applicant's published technique was useful for the implantation of fibroblasts transfected with human granulocyte colony-stimulating factor cDNA into mice to supplement cytokine production by gene therapy. (Tani et al.,

Blood 74(4):1274-1280 (1989) (copy enclosed)). Applicant's work is specifically acknowledged at page 1274, col. 1.

Also in 1989, the merits of Applicant's published technique were recognized and included in a review article entitled "Biotechnology - The Golden Age". (Malik, V. S., *Advances in Applied Microbiology* 34:263-306 (1989) (copy enclosed)). Applicant's work was referred to at page 297 of the article, which appeared in *Advances In Applied Microbiology*.

Ogura et al. reported in 1990 in the journal *Cancer Research* that fibroblasts transfected by calcium phosphate coprecipitation could deliver antitumor α -interferon therapy. (Ogura et al., *Cancer Research* 50:5102-5106 (1990) (copy enclosed)). Once again, tribute is paid to Applicant's published early work. *Id.* at 5102, col. 1.

Teumer et al. also transfected cells using calcium phosphate precipitation to deliver human growth hormone to athymic mice. Teumer et al. used a keratinocyte cell line. Once again, the authors pay tribute to Applicant's technique. (Teumer et al., *FASEB J.* 4:3245-3250 (1990)), at 3245-46 (copy enclosed)).

In 1993, a report of the processing of mutated proinsulin to mature insulin appeared in the journal *Endocrinology*. (Yanagita et al., *Endocrinology* 133:639-644 (1993) (copy enclosed)). The authors reported that they were "currently in the process" of extending this work with the possibility of using it for a hybrid artificial islet or for gene therapy, citing Applicant's 1987 *New England Journal of Medicine* article. (*Id.* at 643, col. 2).

A 1994 article by Rosenthal et al. reports the use of cytokine therapy with gene-transfected cells. The authors reported that the application of G-CSF gene-transfected cells in gene therapy was "currently under investigation in our laboratory", citing the Selden 1987 *Science* and *New England Journal of Medicine* articles. (Rosenthal et al., (Blood 84(9):2960-2965 (1994)), at 2964, col. 2 (copy enclosed)).

Moritani et al. referred to Applicant's published work as part of "the starting basis" that afforded them the opportunity to develop their concept of somatic gene therapy, once again evidence of the adoption of and tribute to Applicant's work. (Moritani et al., (J. Clin. Invest. 98(8):1851-1859 (1996)), at 1858, col. 2 (copy enclosed)).

These reports continue to pay tribute to Applicant's technique and continue to successfully use Applicant's technique to safely transfer various genes into various recipient subjects. In other words, Applicant's work enabled others to successfully transfer various genes into various animals, and it continues to do so. For this reason, Applicant respectfully requests withdrawal of this rejection.

Rejection of Claims 37-71 Under 35 U.S.C. §§ 102(b) and 103(a)

The Examiner rejected claims 37-71 over several references and several combinations of these references. Applicant respectfully traverses these rejections.

I. Those of ordinary skill in the art did not expect ex vivo nonviral gene transfer to work.

Gene transfer, the first step of ex vivo gene therapy, can be carried out in two different ways: (1) viral transduction or (2) nonviral techniques. Viral transduction

involves genetically engineering a viral vector to contain the foreign gene and then exposing cells to the viral vector. Nonviral techniques include chemical techniques, such as calcium phosphate transfection, and physical techniques, such as microinjection, electroporation, and fusion.

As Applicant has discussed, Applicant's invention involves using nonviral transfection and no DNA of retroviral origin to introduce a gene into a cell. None of the references the Examiner cites teach or suggest using nonviral transfection and no DNA of retroviral origin to introduce a gene into a cell.

For example, the Rosenberg patent involves transducing cells by infecting them with a virus. (Rosenberg at col. 2, lines 15-25). Rosenberg does not teach or suggest using nonviral transfection for gene transfer or gene therapy. Furthermore, Rosenberg does not transduce cells to produce insulin, as the Examiner alleges. (Paper No. 10 at 10, lines 29-36). Rather, the cells Rosenberg used contained an insulin gene and expressed insulin before Rosenberg transduced them. (Rosenberg at 3, lines 16-18). Rosenberg's transduction of the cells merely made the cells cancerous so that they could produce larger quantities of insulin over longer periods of time. (Id. at col. 8, lines 7-14).

Similarly, the Goding reference involves fusing cells. Goding does not teach or suggest using nonviral transfection for gene transfer or gene therapy.

As the Examiner states, the Williams et al. and Miller et al. references use retroviruses to introduce a gene into a cell. (Paper No. 10 at 11, lines 8-11, 20-23).

Accordingly, neither the Rosenberg patent, the Goding reference, the Williams et al. reference, nor the Miller et al. reference anticipates the claimed invention because none of these references use nonviral transfection to introduce a gene into a cell. Furthermore, none of the references the Examiner cites, alone or in combination, renders the claimed invention obvious because none of the cited references suggests using nonviral transfection and no DNA of retroviral origin to introduce a gene into a cell.

Prior to Applicant's invention, nonviral techniques had not been used successfully, and those of ordinary skill in the art did not consider them to be suitable for *ex vivo* gene therapy. Those of ordinary skill in the art thought that *ex vivo* gene therapy required a highly efficient method of introducing a gene into a cell and that such high efficiencies could only be obtained with viral transduction, especially retroviral transduction.

A gene therapist summarized the belief those of ordinary skill in the art held in the early and mid-1980s, prior to Applicant's invention. (Transcript of the FDA Biological Response Modifiers Advisory Committee Meeting of December 13, 1990, 51-57 (copy enclosed)). He discussed the inefficiencies of nonviral transfection as a gene transfer method and stated that these inefficiencies had led those of ordinary skill in the art to use retroviral transduction:

So a variety of other techniques have been studied, all of them suffering from this relatively low level of efficiency. So about a dozen, 8 or 10 years ago [about 1978, 1982, or 1980] the field evolved into studying the possibility of using recombinant retroviruses and vectors for transferring genes.

(Id. at 57) .

At the first gene therapy conference in 1983, one of the participants discussed chemical and physical methods of gene transfer and promptly dismissed them all as being inadequate for *ex vivo* gene therapy. (Friedmann, T., Gene Therapy: Fact and Fiction in Biology's New Approaches to Disease 39 (1994) (copy enclosed)). He then went on to discuss the merits of viral transduction for gene insertion. According to him, viral transduction "is, I think, the way to go." (Id. at 40).

In a seminal gene therapy review article in 1984, W. French Anderson discussed various gene transfer methods. (Anderson, at 402, col. 2). Anderson concluded that retroviral transduction is the most promising gene transfer technique:

As noted, retroviral-based vectors appear to be the most promising approach at present for use in humans.

(Id. at 504).

In a review article in 1986, Gilboa et al. discussed nonviral transfection as a gene transfer method and concluded that retroviral transduction is the best gene transfer method for *ex vivo* gene therapy. (Gilboa et al., Transfer and Expression of Cloned Genes Using Retroviral Vectors, 4 *BioTechniques* 504 (1986) (copy enclosed)). Gilboa et al. first dismissed nonviral transfection as a gene transfer method:

Unfortunately, DNA transfection has its limitations. Most significantly, it is a very inefficient means of transferring genes into mammalian cells A second important issue is one of expression--once delivered, how to ensure the proper expression of the gene in the recipient cell.

(Id.). They then discussed the superiority of retroviral transduction as an *ex vivo* gene transfer method:

In the past several years, a new gene transfer technology has emerged which appears to be superior to DNA transfection and other previous techniques and which may offer a new approach to the therapy of human genetic diseases. This new technology is known as retroviral-mediated gene transfer, i.e., the use of retroviruses to deliver genes into cells.

(Id.; internal footnote omitted).

In a 1987 review article, Caskey discussed the inefficiencies of nonviral transfection as a gene transfer method. (C. Thomas Caskey, Genetic Therapy: Somatic Gene Transplants, 22 Hospital Practice 181, 184-85 (1987) (copy enclosed)). According to Caskey, results with non-retroviral transfection showed that "effective gene therapy will require far more efficient techniques of gene transplantation An obvious alternative is to use viruses as vectors, as we use bacteriophage vectors to insert genes into bacteria for cloning." (Id. at 185, col. 1).

Even after Applicant's U.S. filing date, those skilled in the art who did not consider Applicant's invention continued to believe that viral transduction was necessary for *ex vivo* gene therapy. This is in contrast to those references Applicant discussed above that considered Applicant's work and recognized it as novel and pioneering.

For example, in a 1988 review article, Eglitis et al. discussed various gene transfer methods other than viral transduction. (Eglitis et al., Retroviral Vectors for Introduction of Genes into Mammalian Cells, 6 BioTechniques 608 (1988) (copy enclosed)). Eglitis et al. dismissed each of these techniques because "they each suffer from limitations affecting their general applicability." (Id.). Eglitis et al. then discussed

the improved efficiency of retroviral transduction, and they concluded that retroviral transduction is the best gene transfer method for *ex vivo* gene therapy:

Nonetheless, retroviruses still appear to be the best technology so far available for the transfer of clinically relevant genes

(Id. at 613, col. 1).

In a 1989 review article, Kohn et al. also discussed various physical and chemical gene transfer methods. (Kohn et al., Gene Therapy for Genetic Diseases, 7 Cancer Investigation 179, 183 (1989) (copy enclosed)). Kohn et al. dismissed each of these techniques because each "has limitations that make them unsuitable for human gene therapy at present." (Id.). Kohn et al. then devoted the remaining eight pages of the article, including a section devoted to "future prospects," to retroviral vectors. (Id. at 184-91).

In a 1990 review article, Verma also discussed chemical and physical gene transfer methods and dismissed each of these methods as being inefficient. (Verma, I. M., Gene Therapy, Scientific American 68, 69-70 (November 1990 (copy enclosed))). Verma then devoted the remaining six pages of the article to retroviral transduction because "retroviruses are the most promising gene-delivery system supplied thus far" (Id. at 70, col. 1).

In a 1992 article, Rosenberg discussed various chemical and physical methods of gene transfer and noted the low efficiencies of these techniques. (Rosenberg, S., Gene Therapy for Cancer, 268 JAMA 2416, col. 3 (1992) (copy enclosed)). According

to Rosenberg, these low efficiencies had caused those of ordinary skill in the art to turn to viral transduction for gene transfer:

Because of the need for high-efficiency transfer of DNA into cells for clinical applications, attention has increasingly turned to the use of viruses, especially transforming DNA viruses such as papovaviruses, adenoviruses, or more recently murine and avian retroviruses as delivery systems.

(Id.). Rosenberg concluded that "retroviruses, especially those based on the Moloney murine leukemia virus, are now being used in clinical applications." (Id. at 2417, col. 1).

In a 1993 article, Rosenberg et al. stated that, despite the existence of many gene transfer methods, only retroviral transduction was practical:

Although many methods exist for introducing foreign genes into cells, the only method with sufficient efficiency for practical use in human trials involves the use of genetically engineered retroviruses.

(Rosenberg, S.A., et al., The Development of Gene Therapy for the Treatment of Cancer, 218 Ann. Surg. 455 (1993) (copy enclosed)). Indeed, Rosenberg et al.

asserted that *ex vivo* gene therapy was impossible until retroviral vectors had been improved enough to transduce cells at a high efficiency:

The recent development of high-efficiency techniques for gene transduction using retroviruses has made the treatment of human diseases by gene transfer techniques a realistic possibility.

(Id. at 455, col. 1).

Not one of these references after Applicant's filing date considered Applicant's work. Thus, those of ordinary skill in the art did not believe that nonviral gene transfer was feasible. Moreover, unlike those who have considered Applicant's work and

recognized it as pioneering, those who have not considered Applicant's work continue to believe that nonviral gene transfer is not feasible and that viral gene transfer is necessary for *ex vivo* gene therapy.

Notwithstanding the technical obstacles to nonviral gene transfer and the belief of those skilled in the art that these obstacles required the use of viral vectors, Applicant surprisingly discovered a successful method for *ex vivo* gene transfer that does not use a viral vector. Since those of ordinary skill in the art expected that such a method would fail, the cited references do not render the claimed invention obvious.

II. The Kopchick et al. and Salser et al. patent documents do not teach or suggest the claimed invention.

Applicant will specifically address the Kopchick et al. published application and the Salser et al. patent because, unlike the other cited references, neither of these patent documents use a viral vector to transfect cells.

The Salser et al. patent describes removing cells from an animal, transfecting them *in vitro* with a *dhfr* gene, the product of which confers methotrexate resistance, and then introducing the transfected cells into an animal. Methotrexate is then administered to the animal, and, according to the patent, it kills the competing, non-transfected cells, allowing the transfected cells to proliferate. The Salser et al. patent does not teach or suggest the selection and cloning steps of the claimed invention.

Unlike Salser, the claimed invention involves removing cells from an animal, transfecting the cells *in vitro* with a gene, **selecting** the transfected cells expressing the gene *in vitro*, **cloning and expanding** the selected cells *in vitro*, and then introducing

the cloned and expanded cells into an animal. The *in vitro* selection and cloning allow these steps to be carefully monitored and avoid the need to expose an animal to a selective agent, such as the methotrexate of the Salser patent.

Although Kopchick et al. did not use a retroviral vector, they did use retroviral DNA. Kopchick et al. used plasmid pBGH-4 to transfer genes into mouse fibroblasts. They derived plasmid pBGH-4 from plasmid pBGH-3 by removing nonessential regions of Rous sarcoma virus DNA. Plasmid pBGH-3 contains part of the genome of Rous sarcoma virus, including a long terminal repeat region (LTR) with its transcription promoters and enhancers. Thus, Kopchick et al. introduced genes into mouse cells with DNA of retroviral origin.

In addition, neither Kopchick et al. nor Salser et al. teach or suggest using a regulatable promoter, which Applicant recites in dependent claims 82 and 97. Salser et al. do not even discuss promoters. After the failure of Salser et al., those of ordinary skill in the art turned to viral vectors to take advantage of both the infectivity and the natural promoters of viruses.

Kopchik et al. represented as exception in that they did not use a viral vector. They did, however, use the viral promoter of the Rous sarcoma virus. The promoter of the Rous sarcoma virus is a constitutive promoter; it is not a regulatable promoter. Accordingly, like Salser et al., Kopchik et al. do not teach or suggest the claimed gene transfer method.

Conclusion

At the time of Applicant's filing date, those of ordinary skill in the art believed that *ex vivo* gene transfer required viral transduction and, thus, viral vectors. Even now, over ten years after Applicant's filing date, those who are not aware of Applicant's work continue to believe that *ex vivo* gene transfer requires viral transduction and, thus, viral vectors.

Despite this belief, Applicant showed, unexpectedly, that *ex vivo* gene transfer does not require viral transduction, and, thus, it does not require viral vectors. Applicant showed that *ex vivo* gene transfer could be accomplished with nonviral transfection. Applicant even showed that, by not using viral vectors, by selecting cells, and by cloning and expanding the selected cells, the safety and efficacy problems of the prior art could be overcome.

Applicant's showing was so strong that it enabled those who were aware of Applicant's work to successfully practice *ex vivo* gene transfer without using viral vectors. It even led to the founding of TKT, a publicly traded biotechnology company that practices *ex vivo* gene transfer without viral vectors and the oldest surviving gene therapy company.

But TKT is not the only entity practicing *ex vivo* nonviral gene transfer based upon Applicant's work. Numerous researchers continue to practice *ex vivo* nonviral gene transfer and acknowledge Applicant's work as pioneering work that enabled them to practice *ex vivo* nonviral gene transfer.

In view of the extensive evidence of the nonobviousness and enablement of the claimed invention, Applicant respectfully submits that the claims are in condition for allowance. Applicant respectfully requests early allowance of the pending claims so that Applicant's pioneering work will be published in the U.S. in the form of a U.S. patent.

Request for Interview

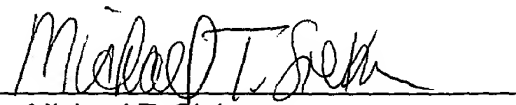
If the Examiner does not believe the claims are in condition for allowance, Applicant respectfully requests the Examiner to contact the undersigned to schedule an interview before the Examiner acts upon this Amendment.

If there are any fees due in connection with the filing of this response, please charge the fees to our Deposit Account No. 06-0916. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested, and the fee should also be charged to our Deposit Account.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Date: March 11, 1998

By: 
Michael T. Siekman
Reg. No. 36,276